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ELECTRON MICROSCOPIC OBSERVATIONS ON FL CELLS INFECTED WITH HERPES SIMPLEX VIRUS I. VIRAL FORMS[1,2]

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SUMMARY FL cells were infected with two laboratory strains (Miyama and HF) and three freshly isolated strains (Iwa, Ko and Watanabe) of herpes simplex virus at an adsorbed multiplicity of approximate 10. The latter three strains were all isolated from patients with *herpes labialis*. Thin sections of cells at late stages of infection were examined by electron microscope. The viral forms of most of the intranuclear particles were 1) capsids with cores of low density, 2) capsids enclosing electron dense cores, 3) empty capsids and 4) granules of 25–30 mμ. The cores varied in shape. Enveloped forms were found in invaginated pockets of the inner nuclear membrane, between the outer and inner nuclear membranes, in cytoplasmic vacuoles and extracellularly. Most enveloped particles had electron dense cores. Besides these characteristic particles several bizarre forms were sometimes encountered. These were 1) capsids enclosing filamentous cores, 2) capsids almost or entirely filled with electron dense material, 3) tadpole-shaped structures and 4) distorted, interwoven membranous structures. Cytopathological changes of host cell organelles were frequently seen. Perichromatin granules and some fragments of the nucleolus were observed. The above findings of characteristic viral forms, bizarre ones and cytopathological changes were common to cells infected with five strains used and presumably these characters are general at least on infection of FL cells with type 1 herpes simplex virus.

INTRODUCTION

Morgan et al. (1953) examined viral particles of herpes simplex by electron microscopy in sections of infected chorio-allantoic membrane. Subsequently numerous electron microscopic investigations have been made on the morphology and development of the virus (Morgan et al., 1959; Wildy et al., 1960; Epstein, 1962a; Watson et al., 1964; Siegert and Falke, 1966; Swanson et al., 1966; Shipkey et al., 1967; Bedoya et al., 1968; Nii et al., 1968a, b, c;

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Schwartz and Roizman, 1969). The negative staining technique unequivocally demonstrated that the virions of herpes simplex virus consisted of three main parts : the envelope, capsid and core (Wildy et al., 1960), while in thin sections of infected cells an enveloped particle enclosing an electron dense core was recognized as a mature form (Morgan et al., 1959 ; Nii et al., 1968a, b). Besides this complete form of virus, various other forms have been observed, especially in thin sections (Morgan et al., 1959 ; Watson et al., 1964 ; Siegert and Falke, 1966 ; Swanson et al., 1966 ; Nii et al., 1966 ; Shipkey et al., 1967 ; Bedoya et al., 1968 ; Rabin et al., 1968 ; Nii et al., 1968a, b ; Schwartz and Roizman, 1969).

Human amnion cells (FL cells) infected with a high multiplicity of the virus showed various forms of virus, and the majority of the particles observed were capsids with a core of either low or high electron density and enveloped particles with a core of high electron density. Moreover, at late stages of infection several aberrant forms of particles were observed (Nii et al., 1966 ; Nii et al., 1968a). However, it was considered that the appearances of these unusual types of particles might be a specific character of the Miyama strain examined and so further experiments were necessary to see whether these previous findings were common to other strains of herpes simplex virus using FL cells as host cells. This paper reports observations on two laboratory strains and three freshly isolated strains.

MATERIALS AND METHODS

For electron microscopy the same specimens were used as in the previous investigation (Nii and Ono, 1971). Briefly, they were prepared as follows. FL cells were infected with two laboratory strains (HF and -GCr Miyama) and three freshly isolated strains (Iwa, Ko and Watanabe) of virus at an adsorbed multiplicity of approximate 10. After 40–63 hr the infected cells were scraped off, precipitated by low speed centrifugation and fixed with 1% glutaraldehyde for 1 hr. Specimens were washed well with Sörensen's phosphate buffer solution and postfixed with 1% osmium tetroxide for 30 min. Then they were dehydrated and embedded in epoxy resin. Sections were stained and examined in a Hitachi 11-B electron microscope as described previously (Nii, 1969 ; Nii and Ono, 1971).

RESULTS

1. *Most common viral forms observed in infected cells*

The viral particles most frequently seen in the nucleus were capsids with cores of low density, capsids enclosing electron dense cores and empty capsids.

The first type had at least two sizes of core. One was a small core of low electron density. Capsids enclosing this type of core were the most frequent in the infected nuclei examined. Fig. 1 shows four such particles (a, b, c and d) which can be distinguished from other particles with electron dense cores. In these four particles an electron translucent area is seen between the shell of the capsid and the opaque core and the center of the core is slightly translucent. Many particles with this type of core are also seen in a viral crystal shown in Fig. 2 although aberrant forms of virus are also present in this crystal. The other type of core is large and consists of a ring-shaped structure of low electron density and a translucent central part, as shown in Fig. 3. In particles with this type of core there is much less space between the two membranous circles of the capsid and the core, than in the former type.

The second particles are capsids enclosing an electron dense core, as shown in Fig. 1. The third particles are empty capsids which are shown by several particles in Fig. 4.

Samples were prepared by synchronous infection of cells with a high multiplicity of virus, but the kinds of viral forms and the frequencies in their appearance varied in different infected nuclei. For example, in the section of two adjacent nuclei shown in Fig. 3 and 4 the morphological appearance of the viral particles were quite different.

Another viral form occasionally seen in the nucleus is granules of 25–30 mμ which seem to have the same composition as the small, low density cores. Fig. 5 shows these granules and three particles with capsids, of which only one (e) is well-defined, surrounded by the reduplicated nuclear membrane.

Lastly, enveloped particles which are the most developed form of herpes virus are shown in Fig. 6 and 7. Fig. 6 shows three enveloped particles located in indented pockets of the inner nuclear membrane and one particle (arrow) in process of envelopment. Reduplication of the nuclear membrane is remarkable. Fig. 7 shows two, extracellular enveloped particles.

2. *Bizarre viral forms*

Several aberrant viral forms were also found. The particles shown by arrows in Fig. 8 are almost entirely filled with fragmented or amorphous electron dense material. A few empty capsids are also seen. One of the two particles in the nucleus in Fig. 9 seems to be completely packed with electron dense material. One tadpole-shaped particle is seen in the space between the outer and inner nuclear membranes in Fig. 10. The tail-like structure is connected to an electron dense core and the particle seems to be completely enveloped.

Bizarre capsid-like material was seen in cells infected with all five strains. Fig. 11 shows a part of a nucleus infected with the Ko strain. In the center interwoven membranous structures and a few particles with capsids and cores of low density are seen surrounded by amorphous electron dense material, i.e. fragmented amorphous parts of the nucleolus. Bizarre forms are also seen in the lower left of the figure. Fig. 12 shows a large aggregation of these structures in a nucleus infected with the HF strain, while Fig. 13 shows a part of a nucleus infected with the Watanabe strain where a few bizarre forms are seen with particles with capsids and cores of low density.

3. *Comparison of the electron densities of viral cores of particles in the nucleus (N) and cytoplasm (C)*

In the nucleus shown in Fig. 14 there are at least eight capsids with cores of low density and eight with electron dense cores. On the contrary in the cytoplasm only the two particles shown by arrows have cores of low density, the rest having electron dense cores.

All section of infected cells examined showed the same tendency.

4. *Morphological changes of host cell organelles*

Disintegration of the nucleolus and condensation of chromatin are characteristic cytopathological changes on herpes virus infection. A disintegrated nucleolus is shown in the center of Fig. 15. Two structures can be differentiated: one (nucleol A) consists of electron dense amorphous material and the other (nucleol B) appears filamentous. Condensed chromatin is also seen in the left, upper part of this figure. In Fig. 16 perichromatin granules (arrows) are conspicuous. In Fig. 17 many spherical particles (arrows) which are extremely electron dense and variable in size are distributed throughout the nuclear area. The origin of these structures is unknown. Interchromatin granules (ICG) and two other kinds of granules (gr A and gr B) thought to be host cell organelles are also seen.

DISCUSSION

FL cells infected with two laboratory strains (HF and –GCr Miyama) and three freshly isolated strains (Iwa, Ko and Watanabe) of herpes simplex virus were observed by electron microscopy and different forms of virus were seen.

Viral cores differed in size and electron density.

At least two types of core of low electron density were recognized, large and small (Fig. 1 and 3). These two types were also seen in previous work on the effect of hydroxyurea on the development of herpes simplex virus (Nii

et al., 1968b). Similar observations were made by Schwartz and Roizman (1969) who named these two types of core, translucent and electron opaque. The possibility that capsids with a large core of low electron density are young forms of particles with a small core is only hypothetical.

Similarly, two or three forms of electron dense core can be differentiated. Several dense cores found in the nucleus in Fig. 1 are large and are round or oval. Almost all the particles found extracellularly or in the cytoplasm have this type of core. On the other hand the dense cores in Fig. 8 and 15 are rod- or ring-shaped.

The electron dense cores contain viral DNA judging from the following results. 1) On treatment of thin sections of infected cells with DNase the electron dense cores disappeared (Epstein, 1962b). 2) Electron dense cores gave a positive Feulgen reaction while cores of low density gave a negative reaction (Peters, 1966). 3) Addition of hydroxyurea to the medium prevented formation of dense cores but cores of low density were produced (Nii et al., 1968b).

In the normal course of infection most viral particles which egress from the nucleus have an electron dense core whether they are enveloped or not, while in the nucleus most particles have cores of low density (Watson et al., 1964; Nii et al., 1968a). There are at least two possible explanations of this. First, cells may have a function of selective transport by which only capsids with electron dense cores are transferred from the nucleus to the cytoplasm. Second, although most particles in the nucleus have cores of low density these cores acquire density just before the particles leave the nucleus, especially near or at the inner nuclear membrane. Further work is required to see if either of these is the actual explanation.

The shell of capsids enclosing electron dense cores tends to be slightly thinner than that of particles with cores of low density (Fig. 1), suggesting a difference in the configurations of the capsomeres in these particles. This also requires further study.

Accumulations of granules (25–30 mμ) thought to have the same composition as the viral cores of low electron density are frequently seen in the nucleus (Fig. 5). These granules were first described by Morgan et al. (1959) and subsequently by several other investigators (Siegert and Falke, 1966; Nii et al., 1966; Nii et al., 1968a; Rabin et al., 1968; Schwartz and Roizman, 1969). Presumably these granules are not the primary form which is later enclosed in the shell of capsids but represent excess production of core material. Correlated with these granular aggregates Swanson et al. (1966) observed plexiform vermicellar arrays with thicker section. These structures were not seen in the present investigation.

Various bizarre viral forms of herpes simplex virus were often found in FL cells infected with this agent. First, capsids partially filled with electron dense material (Fig. 8) or those packed with electron dense material (Fig. 9) were sometimes seen (Nii et al., 1968a; Nii, 1969). Viral particles with filamentous cores were found by Swanson et al. (1966) in a biopsy specimen from the brain of a patient with *herpesvirus hominis* encephalitis. Similar particles are seen in Fig. 2 in this report. This indicates that virus cores with varied morphology are not uncommon in cells infected with herpes simplex either in vivo or in vitro. Second, capsids associated with tail-like structures were occasionally seen in the nucleus (Fig. 14) and enveloped forms of these particles were also observed (Fig. 10). These tadpole-shaped structures of herpes simplex virus were first described by Nii et al. (1968a), and the structures have also been encountered in studies on various viruses of the herpes group, namely, Lucké adenocarcinoma (Fawcett, 1956), equine rhinopneumonitis virus (Sharp and Bracken, 1960), cytomegalovirus (Luse and Smith, 1958; Ruebner et al., 1965), feline rhinotracheitis virus (Ditchfield and Grinyer, 1965) and varicella-zoster virus (Cook and Stevens, 1970). Third, distored, interwoven membranes (Fig. 11, 12 and 13) were produced with all the five strains tested and the membranes were sup-

posed to result from aberrant capsid differentiation. They were always encountered in FL cells at late stages of infection regardless of the viral multiplicity at infection (unpublished data) but their appearance was greatly dependent upon the kind of host cell. Thus the interwoven membranes were less frequently observed with BHK cells and were not observed with Earle's L cells (Nii et al., 1966).

Some cytopathological changes of host cell organelles, related with disintegration of the nucleolus and condensation of chromatin are also described in this report. Perichromatin granules or dense granules were conspicuous (Swanson et al., 1966; Shipkey et al., 1967; Bedoya et al., 1968) and various modifications of the structure of the nucleolus were also observed (Swanson et al., 1966; Sirtori and Bosisio-Bestetti, 1967; Bedoya et al., 1968; Schwartz and Roizman, 1969). Presumably these cytopathological changes are not directly due to synthesis of herpes simplex virus but to disintegrative changes of the host cell organelles.

In this investigation, the appearance of various viral forms and characteristic cytopathological changes of FL cells were encountered not only with the Miyama strain of herpes simplex virus but also with another laboratory strain (HF) and three freshly isolated strains from patients with *herpes labialis* (Iwa, Ko and Watanabe). Experiments are now in progress to see whether there are any differences in the viral forms of Type 1 and Type 2 herpes simplex virus in infected FL cells.

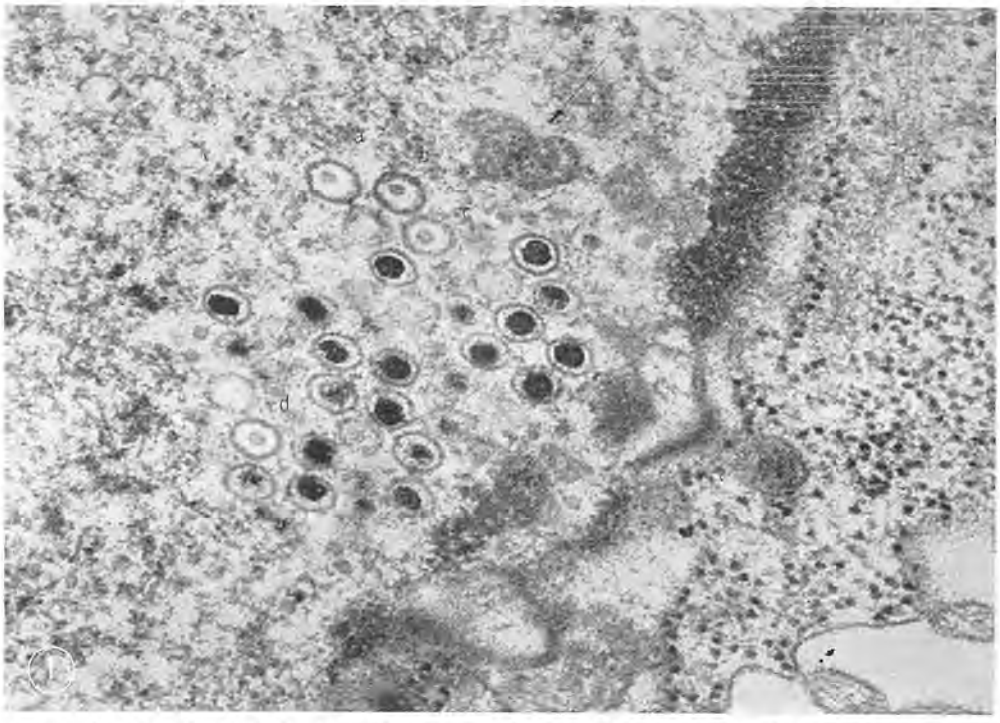


FIGURE 1. *An FL cell 44 hr after infection with the –GCr Miyama strain of herpes simplex virus. An aggregate of capsids which either contain cores of low density or electron dense cores is located in a part of the nucleus adjacent to the nuclear membrane. A structure of bizarre shape (indicated by an arrow) is also seen.* × 75,000

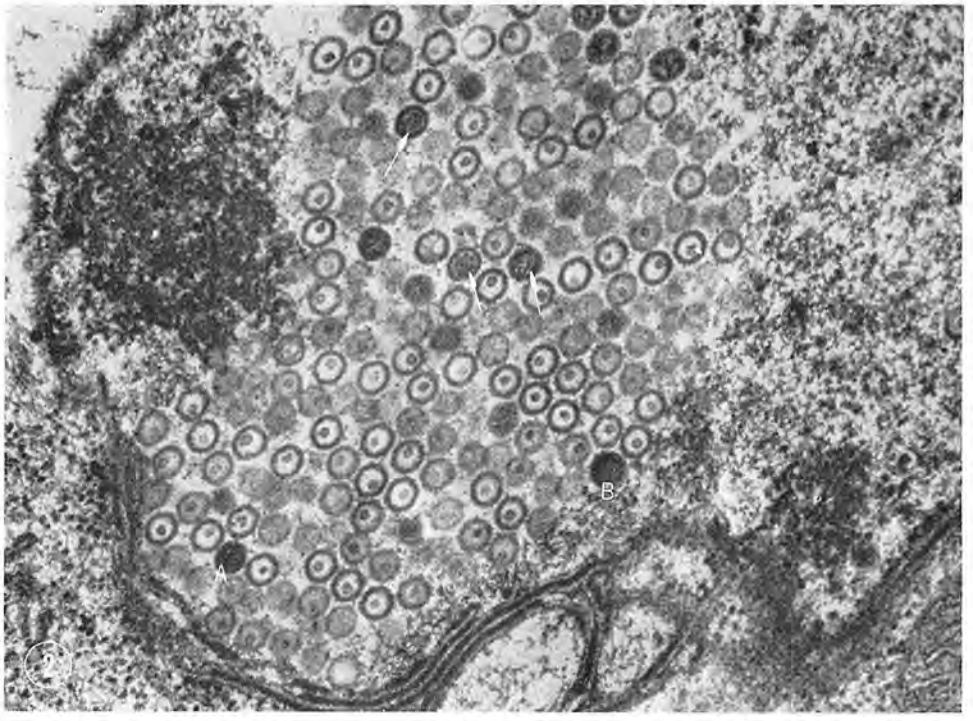


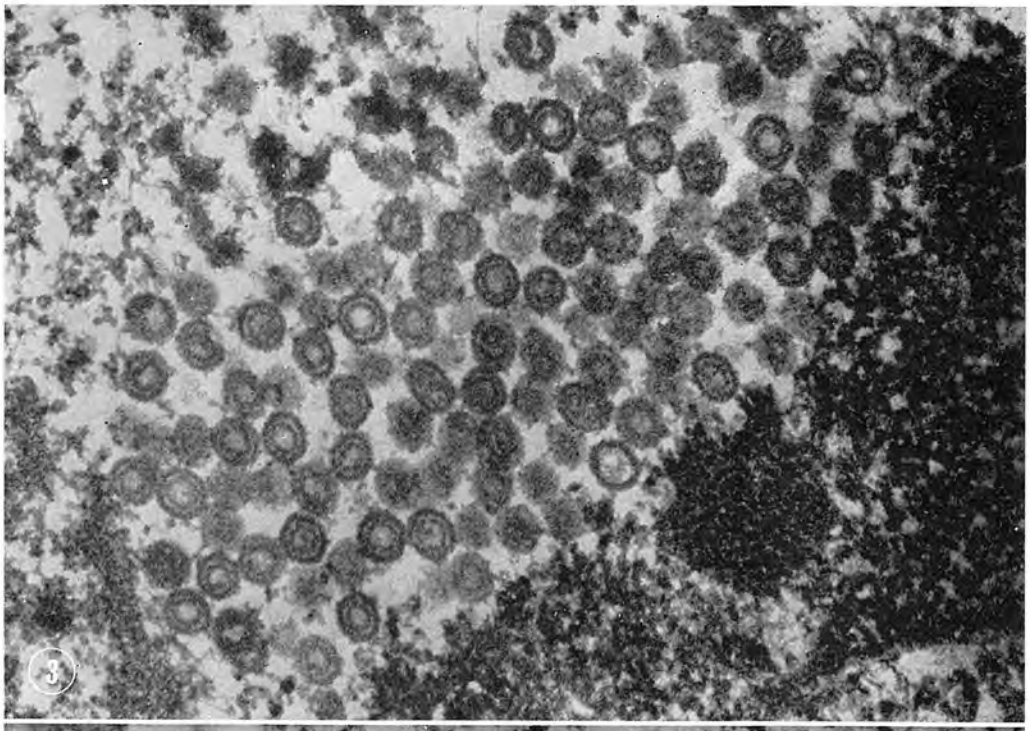
FIGURE 2. *A viral crystal in the nucleus of an FL cell 63 hr after infection with the Watanabe strain of herpes simplex virus. Most of the particles in the crystal are capsids with small cores of low density. Cores of particles indicated by arrows are filamentous. Particles A and B are filled with electron dense material.* ×*48,000*

▶

FIGURE 3. *An aggregate of capsids with large, ring-shaped cores in the nucleus of an FL cell40 hr after infection with the –GCr Miyama strain of herpes simplex virus. The centers of the cores are translucent.* ×*70,000*

▶

FIGURE 4. *Viral particles in the nucleus of an FL cell 40 hr after infection with the –GCr Miyama strain of herpes simplex virus. Several capsids are empty and some capsids have small cores of low electron density. One particle in the lower, middle part has an electron dense core.* ×*52,000*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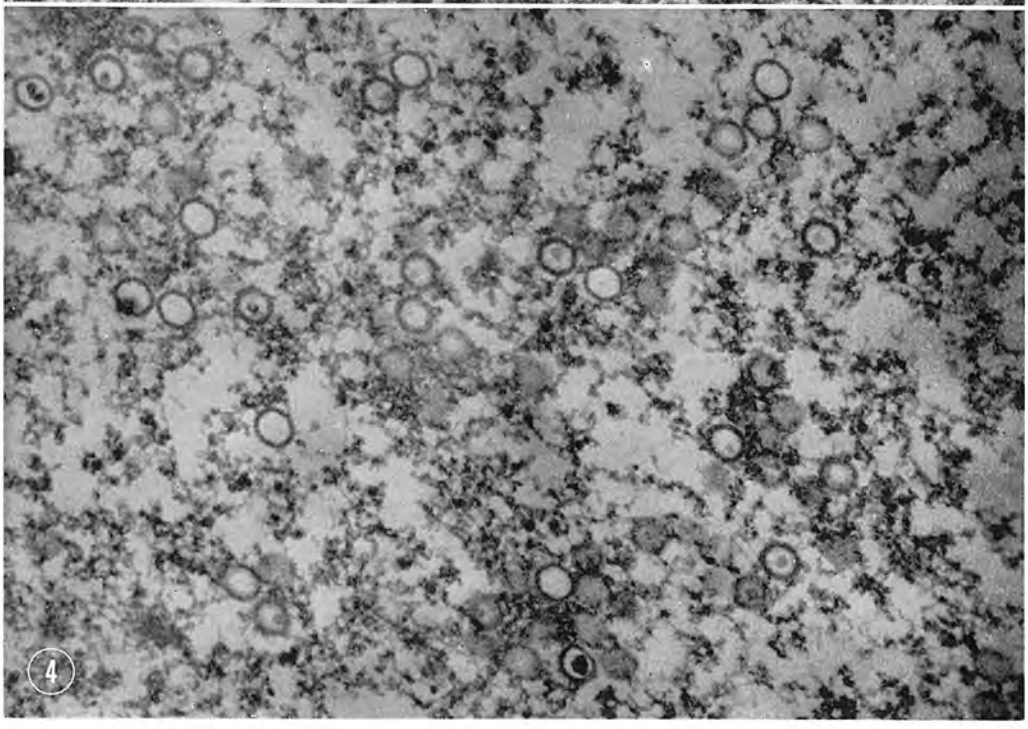
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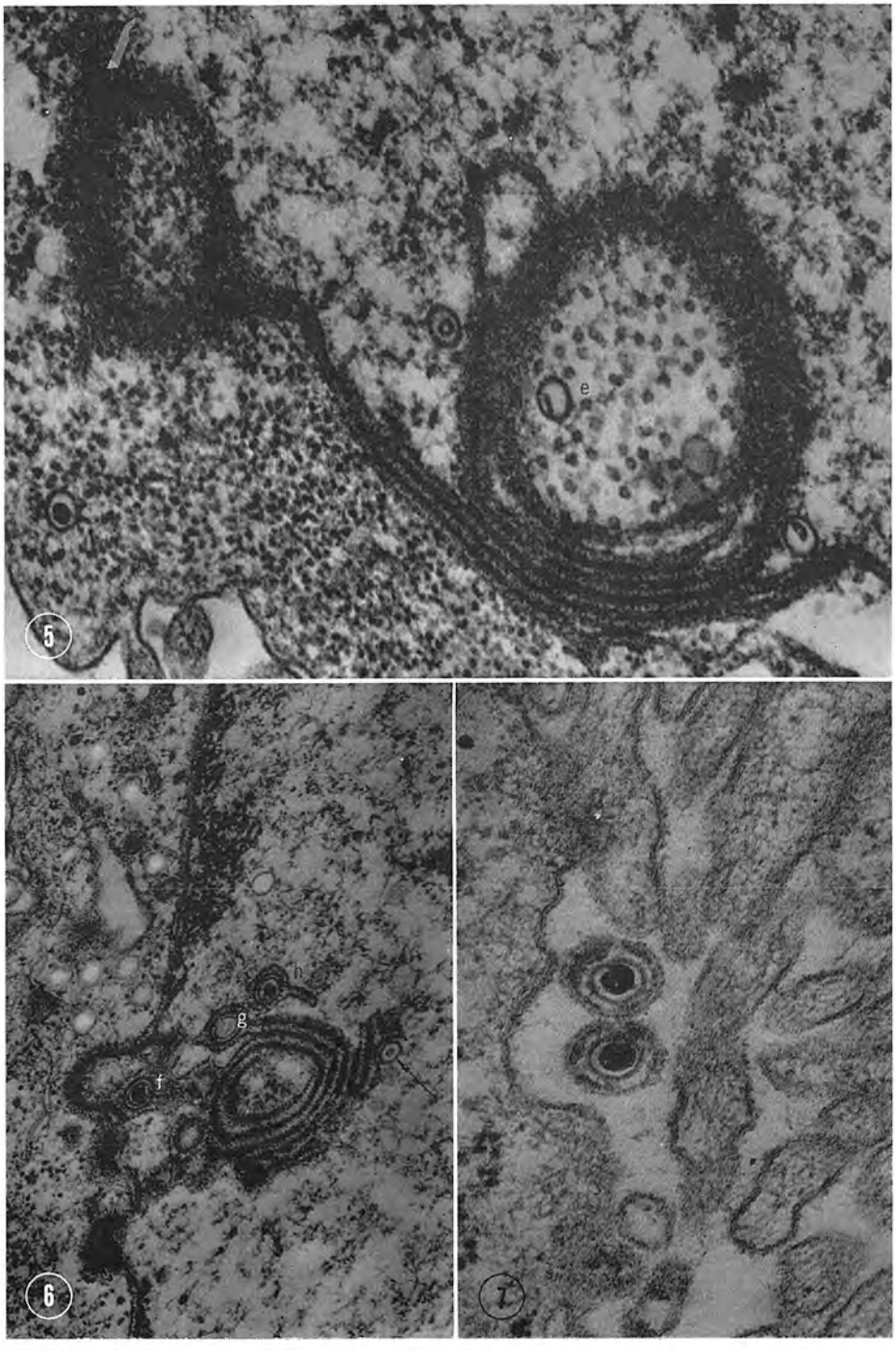
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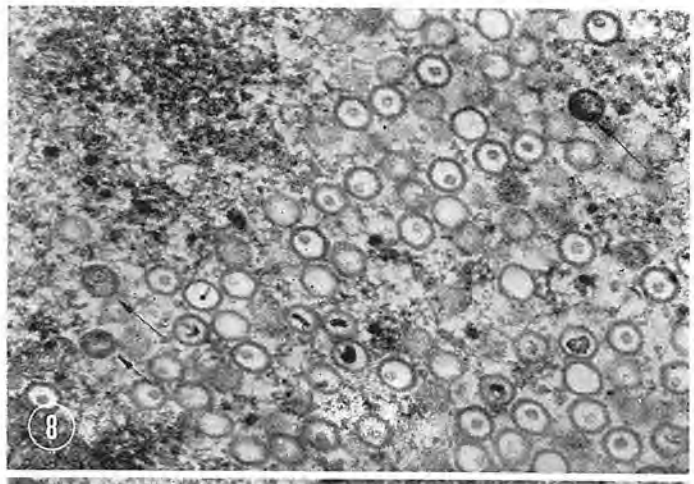
FIGURE 5. *Part of an FL cell 63 hr after infection with the Watanabe strain of herpes simplex virus. Granules of 25–30 mμ and three capsids including a well defined particle (e) are surrounded by reduplicated nuclear membrane. A capsid with an electron dense core is seen in the cytoplasm.* ×*63,000*

FIGURE 6. *Part of an FL cell 62.5 hr after infection with the Iwa strain of herpes simplex virus. Three enveloped particles (f, g and h) are each in an indented pocket of the inner nuclear membrane and one particle (arrow) is in the process of budding. Reduplication of the nuclear membrane is remarkable.* ×*43,000*

FIGURE 7. *Two enveloped particles in the extracellular space between FL cells 44 hr after infection with the –GCr Miyama strain of herpes simplex virus.* ×*76,000*



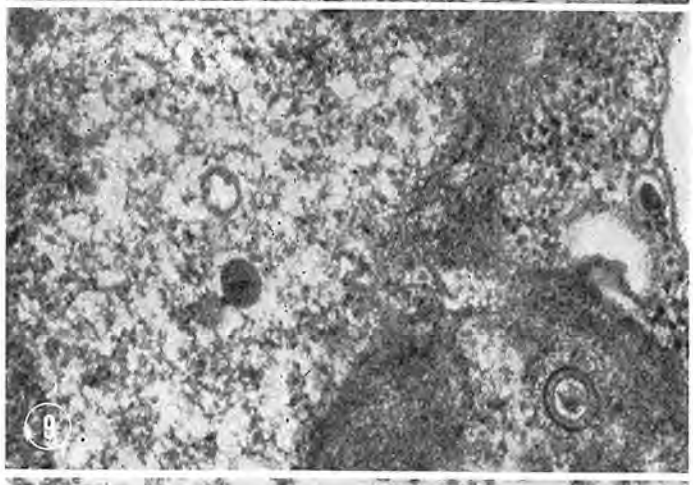


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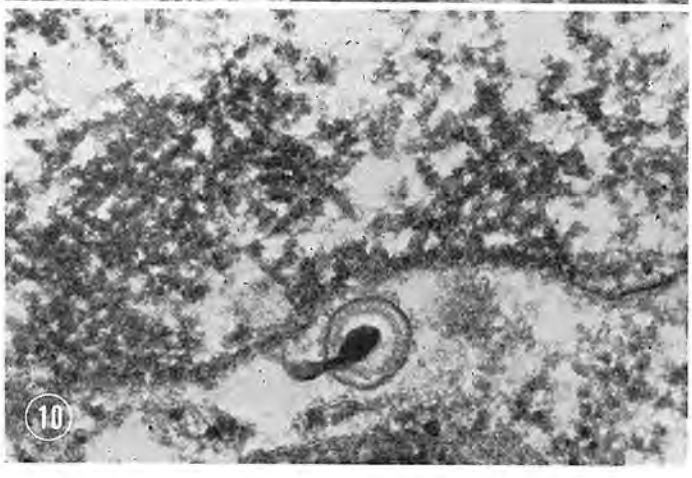
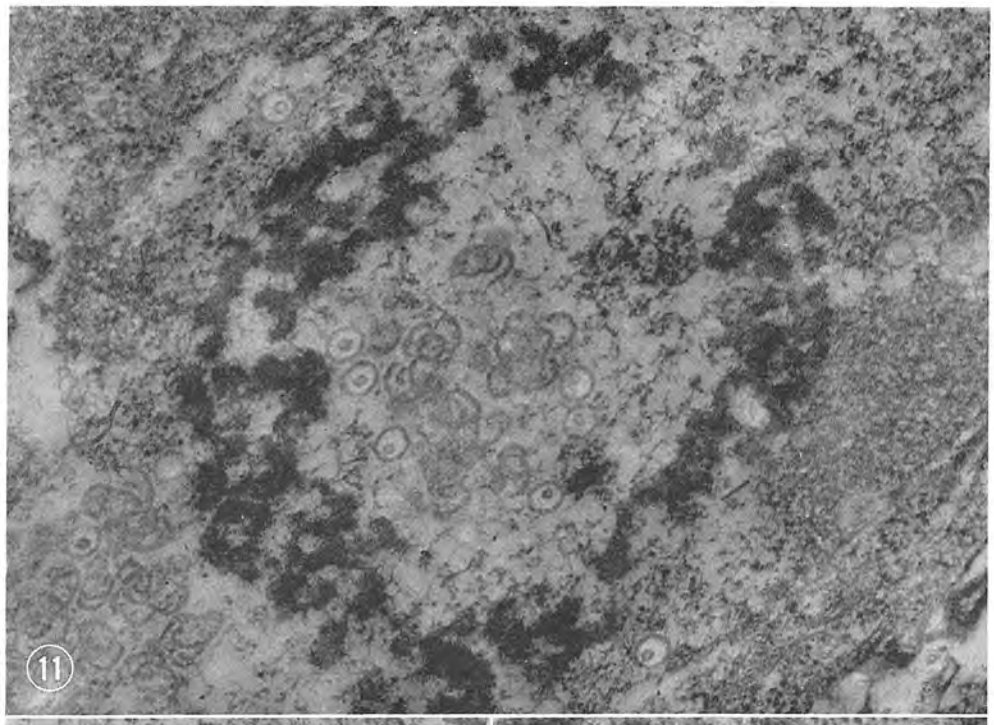
FIGURE 8. *An aggregate of viral particles in the nucleus of an FL cell 43 hr after infection with the HF strain of herpes simplex virus. Most of the particles indicated by arrows are filled with electron dense material.* ×*60,000*

FIGURE 9. *Part of an FL cell 62 hr after infection with the Ko strain of herpes simplex virus. One particle in the nucleus is packed with electron dense material.* ×*65,000*

FIGURE 10. *A tapdole-shaped particle between the outer and inner nuclear membranes of an FL cell 40 hr after infection with the Miyama strain of herpes simplex virus. The tail-like structure is connected to the electron dense core of the particle.* ×*100,000*

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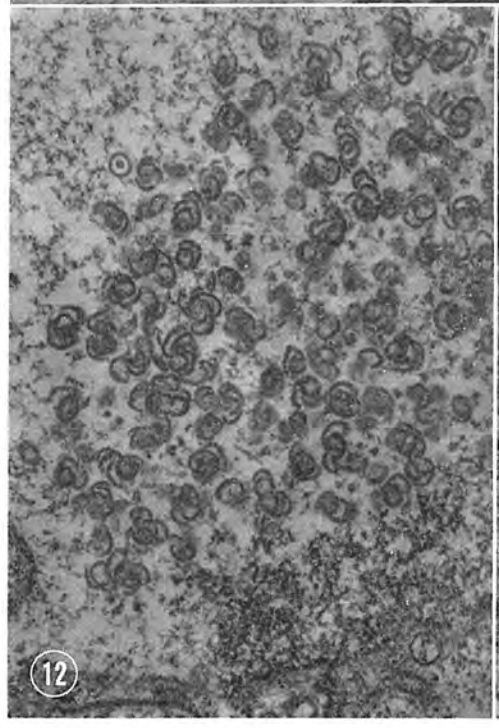
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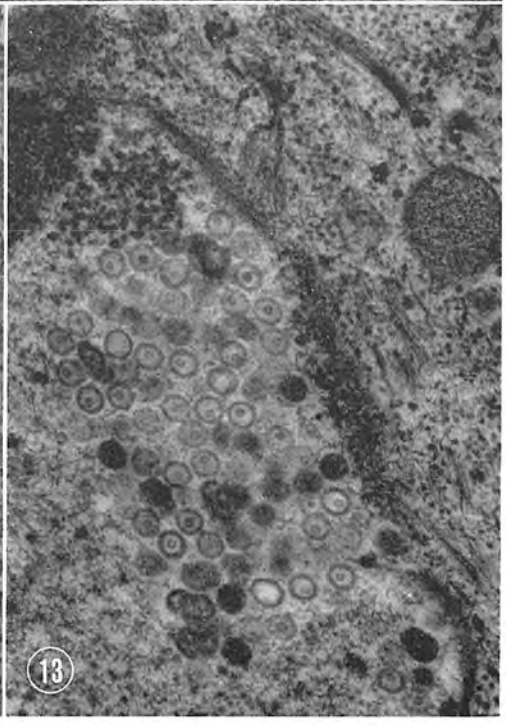
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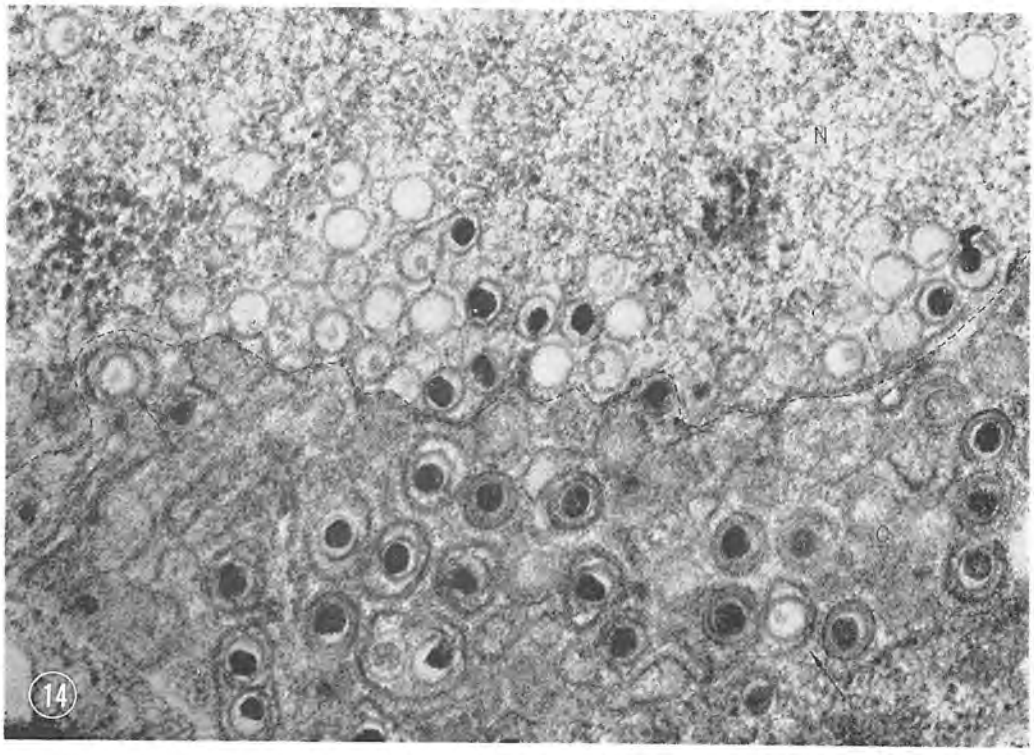
FIGURE 11. *Bizarre interwoven membranes in the nucleus of an FL cell 62 hr after infection with the Ko strain of herpes simplex virus. Fragmented electron dense material derived from the nucleolus surrounds six capsids with cores of low density and an aggregate of interwoven membranous structures. These structures are also seen at the left, low corner of this picture.* ×51,000

FIGURE 12. *Aggregates of interwoven membranous structures in an FL cell 43 hr after infection with the HF strain of herpes simplex virus.* ×35,000

FIGURE 13. *An aggregate of viral particles adjacent to the nuclear membrane in the nucleus of an FL cell 63 hr after infection with the Watanabe strain of herpes simplex virus. Some viral particles with bizarre forms are also seen, i.e. a few small aggregates of interwoven membranous structures and several particles packed with electron dense material.* ×36,000

FIGURE 14. *Viral particles in an FL cell 62 hr after infection with the Ko strain of herpes simplex virus. In the nucleus (N) capsids either containing cores of low electron density or appearing empty are more numerous than capsids containing electron dense cores. In the cytoplasm (C) all except two particles (arrows) have electron dense cores.* ×65,000



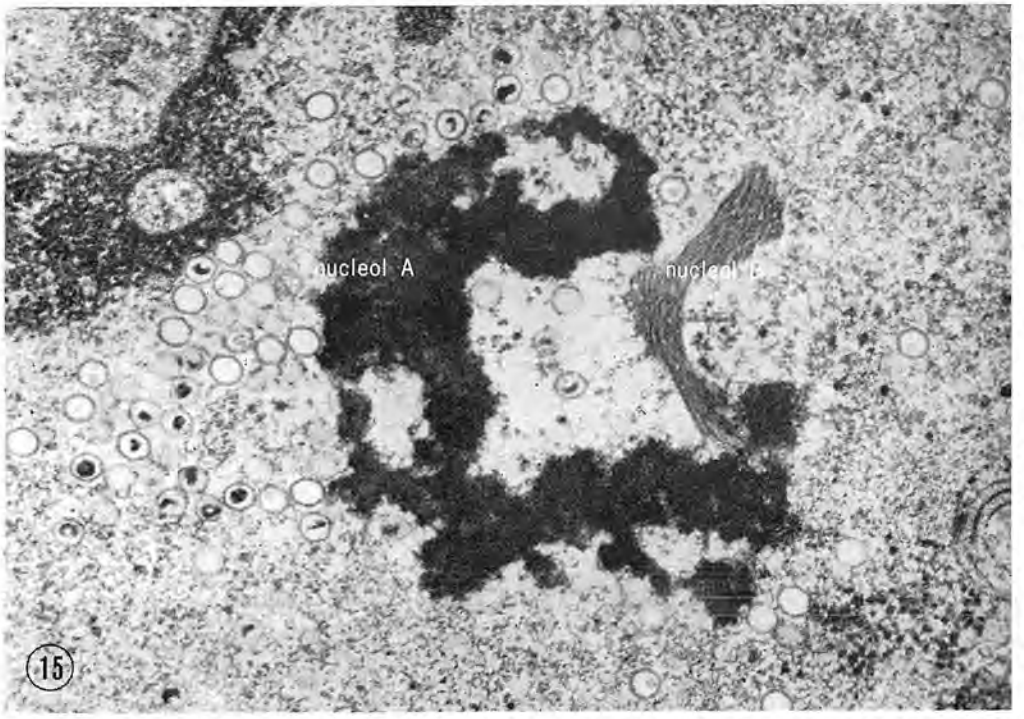


FIGURE 15. *Part of the nucleus of an FL cell 43 hr after infection with the HF strain of herpes simplex virus. Disintegrated fragments of the nucleolus are seen as amorphous electron dense material (nucleol A) and a bundle of filaments (nucleol B). Condensation of chromatin is also evident in the left upper part of this picture.* ×46,000

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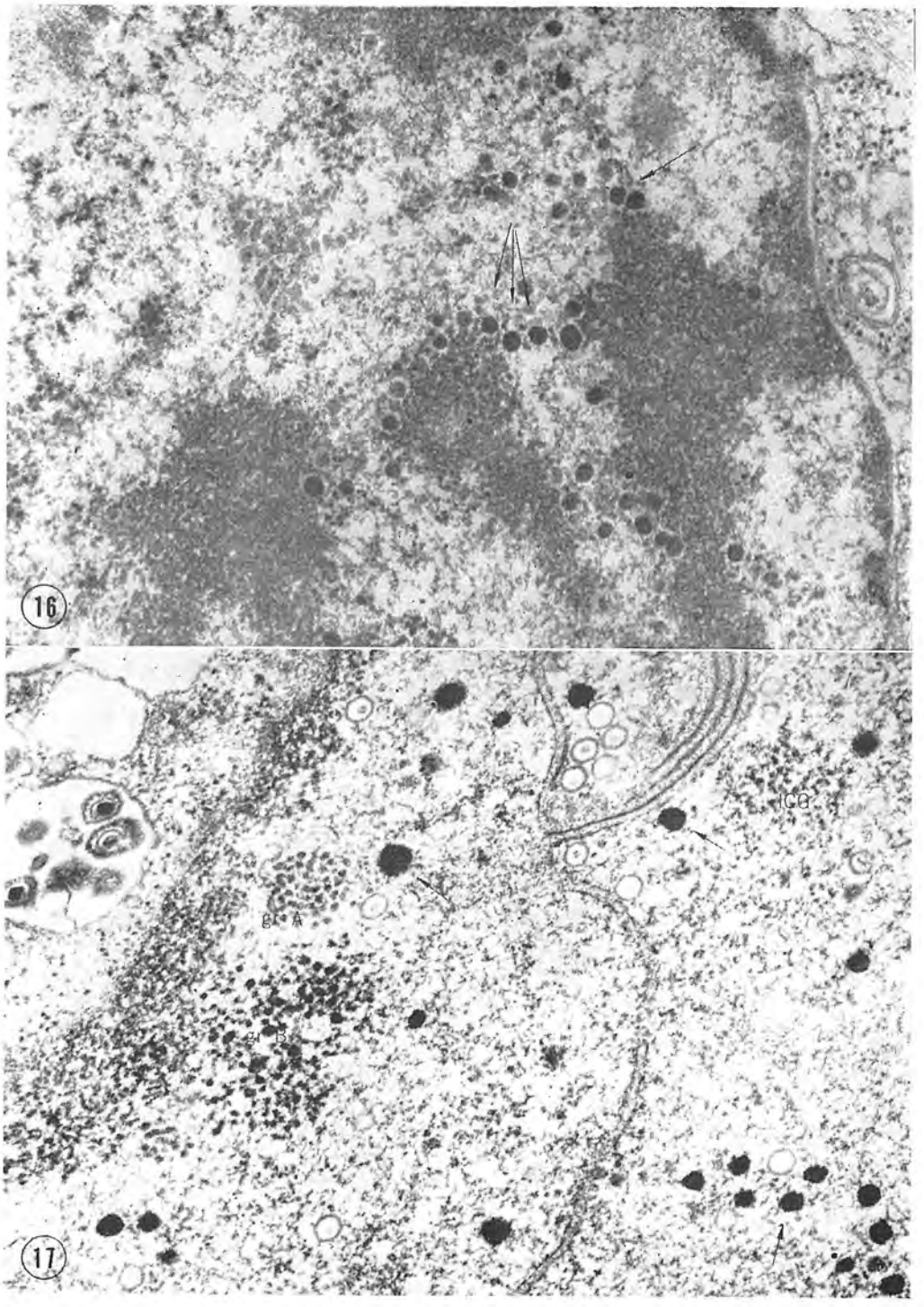
FIGURE 16. *Part of the nucleus of an FL cell 44 hr after infection with the Miyama strain of herpes simplex virus. Electron dense perichromatin granules are conspicuous.* ×53,000

FIGURE 17. *Part of an FL cell 43 hr after infection with the HF strain of herpes simplex virus. Many electron dense, spherical particles are seen in the nuclear area. Interchromatin granules (ICG) and two kinds of granules (grA and grB) are also seen. Viral capsids in the nucleus are either empty or contain cores of low density while three enveloped particles in a cytoplasmic vacuole have electron dense cores.* ×38,000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